Aging-related impairment of urine concentrating mechanisms correlates with dysregulation of adrenocortical angiotensin type 1 receptors in male Fischer rats

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To investigate age-associated impairments in fluid homeostasis, 4-months (young) and 32-months (old) Fischer 344/BN male rats were studied before and after a dietary sodium load. Transferring young rats from a low-sodium (LS) to a high-sodium (HS) diet increased water intake and urine volume by 1.9- and 3.0-fold, respectively, while urine osmolality and plasma aldosterone decreased by 34% and 98%. Concomitantly, adrenocortical angiotensin-type-1-receptor (AT1R) density decreased by 35% and AT1bR mRNA decreased by 39%; no changes were observed in AT1aR mRNA. In contrast, the increase in water intake (1.4-fold) was lower in the old rats and there was no effect of the HS diet on urine volume or urine osmolality. AT1R densities were 29% less in the old rats before transferring to the HS diet and AT1R densities were not reduced as rapidly in response to a HS diet compared to the young animals. After six days on the HS diet, plasma potassium was lowered by 26% in the old rats, whereas no change was detected in the young rats. Furthermore, while plasma aldosterone was substantially decreased after 2 days on the HS diet in both young and old rats, plasma aldosterone was significantly lower in the old compared to the young animals after two weeks on the LS diet. These findings suggest that aging attenuates the responsiveness of the adrenocortical AT1R to a sodium load through impaired regulation of AT1bR mRNA, and that this dysregulation contributes to the defects in water and electrolyte homeostasis observed in aging.
Individuals 65 years and older are one of the most rapidly growing segments of the United States population. Changes in the control of sodium and water balance is a major characteristic of the normal human aging process and includes a decrease in thirst, urinary concentrating ability and capacity to excrete water and electrolytes (32). These age-related changes in humans are also observed in animals. Aging impairs the ability of rats to excrete a sodium load (11) and to maximally concentrate urine (12). These changes in fluid and electrolyte regulation can put the elderly at increased risk for disorders of hyponatremia (due to water retention) or hypernatremia (as a result of sodium retention), which can cause central nervous system dysfunction and also negatively impact medication effectiveness, resulting in adverse clinical events and surgical outcomes as well as other physiological functions (34, 38).

The adrenal steroid hormone aldosterone plays a key role in the homeostatic mechanisms controlling fluid and electrolyte balance (28, 40). In humans (22, 62) and experimental studies of animal models (9), aging is associated with decreased plasma aldosterone levels (10, 47). Aging-related changes in aldosterone are magnified under conditions that stimulate aldosterone secretion indicating that not only is plasma aldosterone reduced in the old, the aldosterone responsiveness to appropriate stimuli is diminished. Sitting upright increases plasma aldosterone in both young adult and old individuals but the magnitude of this increase is smaller in the elderly (35, 54, 65). Likewise, when sodium intake is restricted or plasma volume is reduced, plasma aldosterone levels rise to a greater degree in young adult compared to old individuals (14, 63).

Aldosterone is synthesized in adrenal glomerulosa cells within the adrenal cortex and secretion of this hormone is regulated by sodium, potassium, adrenocorticotropic
hormone and angiotensin II (Ang II). One likely contributor to the aging-associated
decrease in plasma aldosterone is an attenuation in adrenal responsiveness to Ang II
since Ang II is the major controller of aldosterone production when dietary sodium is
altered (30). Ang II infusion into young adult 8-10 months (mo) of age and old (28-32 mo)
Long-Evans rats increased plasma aldosterone; however, the response to Ang II was
significantly smaller in the old rats compared to the young adult rats (50). These
findings are not restricted to rodents as Ang II-induced aldosterone production was
lower in adrenal glomerulosa cell suspensions from old cows compared to those from
young cows (49).

Aldosterone release from the adrenal cortex is primarily mediated by activation of
the angiotensin type 1 receptor (AT\textsubscript{1}R). Many studies in young adult animals have shown
that the adrenal AT\textsubscript{1}R plays a key role in maintaining electrolyte balance in response to
changes in dietary sodium. A HS diet down-regulates adrenal AT\textsubscript{1}R expression and
aldosterone release while a LS diet has the reverse effects (2). What is not well known is
how aging alters the adrenal AT\textsubscript{1}R response to dietary sodium manipulation. This study
investigated the regulation of adrenocortical AT\textsubscript{1}R protein and mRNA during the
adaptation response to a sodium load as a function of age to increase our understanding of
the mechanisms influencing age-associated impairments in fluid homeostasis. To maximize
the sodium load, we maintained the rats on a LS diet for two weeks before transferring
them to a HS diet. We chose the Fischer 344/BN rat to avoid the confounds of age and
sodium-induced hypertension since these animals remain normotensive throughout their
life-span (7) and their blood pressure increases only marginally on a HS diet (16).

Materials and Methods

Animals. Male Fischer 344BN rats at 4 months (young) and 32 months (old)
of age were purchased from the National Institutes of Aging and individually housed in a temperature-controlled animal facility. All rats were maintained on a LS (0.13% NaCl) diet for two weeks. Subsequently, all rats were then transferred to a HS (4% NaCl) diet for up to 6 days (Teklad, Madison, WI). The animals were given tap water ad libitum under controlled conditions (12 hrs light/dark schedule at 24°C). Body weight (BW) was measured daily. Animals were placed in metabolic cages for determination of daily water and food intake and collection of urine. Under isoflurane anesthesia, the adrenal was removed, trunk blood was collected by cardiac puncture and the animals were euthanized by exsanguination. The Georgetown University Animal Care and Use Committee approved all procedures.

Urine and plasma analysis. Urine osmolality (osm) was measured by freezing point depression (Model 3900 osmometer; Advanced Instruments Inc., Norwood, MA). Plasma was collected from heparinized trunk blood and plasma sodium and potassium were determined by Easylyte Na/K Analyzer (MEDICA Corporation, Bedford, MA). Plasma aldosterone was measured by RIA (Coat-a-count, Siemens, Los Angeles, CA). Plasma vasopressin (AVP) content was measured by radioimmunoassay after extraction, as previously described (56, 64).

AT₁R radioligand binding. Membranes were prepared from the adrenal cortex as described previously (25, 67). Membranes (5-10 µg protein/tube) were incubated for 1-2 h at room temperature with increasing concentrations of the Ang II antagonist ¹²⁵I-[Sar¹,Ile⁸]Ang II in the presence of 1 µM PD-123319, an AT₂R antagonist to ensure only AT₁R expression was measured (67). ¹²⁵I-[Sar¹,Ile⁸]Ang II was prepared as previously described (60). Binding reactions were terminated by rapid filtration through a Brandel cell harvester. Specific AT₁R binding was determined by the total amount of radioligand bound minus nonspecific binding (defined as the amount bound in the presence of 200 nM
Ang II, i.e., 100 x Kd for Ang II). Data points were obtained in triplicate. Kd and Bmax values were determined using the one-site saturation binding nonlinear regression analysis program, PRISM (GraphPad Software, Inc).

Real-time PCR: Total RNA from adrenal cortex was extracted using TRIzol reagent (Life Technologies). First strand cDNA was made from total RNA using iScript cDNA synthesis kit (BioRad, Hercules, CA) with MMLV RNase H+ reverse transcriptase, oligo(dT) and random hexamers. Quantitation of specific mRNAs and 18S rRNA (for control) were performed by real-time PCR using the ABI Prism 7700 Sequence Detection System (Applied Biosystems Inc., Foster City, CA) (66). The PCR reaction mixture consisted of RNase free water, TaqMan Universal PCR Master Mix (Perkin Elmer Applied Biosystems) and 300 nM specific primers and 10 μM probe (For AT1aR primers and probe: Forward primers: 3'UTR-47F, 5'-GCA GCC TCT GAC TAA ATG GCT T-3'; Reverse primer: 3'UTR-191R, 5'-CAA GAC GGC TTT GCT TGG TTA-3'); and Probe: 3'UTR-70T, 6 FAM-CGA CCA AAG GAC CAT TCA CCC TGC-TAMRA. For AT1bR primers and probe: Forward primers: 3'UTR-38F, 5'-AGC AGA AGC CAG AGG ACC ATT-3'; Reverse primer: 3'UTR-142R, 5'-CAC TGA GTG CTT TCT CTG CTT CA-3'; and Probe: 3'UTR-89T, 6 FAM-AGT GTT CAA CCT CCA GCA ATC CTT TCA GG-TAMRA) and cDNA samples. PCR conditions were optimized for the 2 probes (232T and 89T) and sets of primers (47F & 191R and 38F &142R) using control cDNAs. The specificity of these primers was confirmed using the AT1R and AT1bR in pCR3 (Invitrogen, Grand Island, NY); we did not detect any amplified products using AT1aR specific primers in the AT1bR expressing cells and vice versa. The expression of 18S rRNA, AT1aR and AT1bR mRNA in each sample was quantitated using the specific primers specified above. PCR reactions without reverse transcription were included to control for contamination by genomic DNA. The standard curves for 18S rRNA, AT1aR and AT1bR mRNA were made
from a series of ten times dilutions ($5^3$, $5^4$, $5^5$, $5^6$, $5^7$, and $5^8$) for each cDNA. The tissue levels of these cDNAs were calculated based on the standard curves.

**Statistics:** Data are expressed as means ± SEM and in some cases as the ratio of the parameters measured under HS and LS dietary conditions at day zero. Statistical significance of the differences between groups was assessed by Student's t-test and two-way ANOVA. Differences were considered significant at p<0.05.

**Results**

**Body Weight**

Young male rats were approximately half the BW of old rats (Fig. 1A). Switching from a LS to a HS diet reduced BW in the old but not the young rats (p<0.0001, young vs old by two-way ANOVA) (Fig. 1A&B). When the data were normalized to BW on day zero of the HS diet, the BW of the old rats dropped by 12% on day 2 and remained reduced on day 6 (Fig. 1B).

**Water and Food Intake**

Young rats rapidly increased their water intake by 1.9-fold on day 2 after switching to a HS diet and remained increased on day 6 (Fig. 2A&B). Water intake on the LS diet was 51% less in the old rats and remained lower after switching to a HS diet compared to the young rats (p<0.0001, young vs old by two-way ANOVA) (Fig. 2A). In response to the HS diet, the old rats also increased their water intake though in a slower manner compared to the young rats; water intake increased by 1.4-fold on day 2 and by 1.9-fold on day 6 (Fig. 2B).

Young rats responded to the HS diet by decreasing their food intake by 30% on days 2 & 6 (Fig. 2C&D). Old rats ate 53% less food than the young rats on the LS diet (Fig. 2C),
which accounts for the reduced body weight gain compared to the young rats. In response to a HS diet, the old rats also decreased their food intake but to a greater extent (52% inhibition on day 2) than the young rats (p<0.01, young vs old by two-way ANOVA).

There was no difference in the NaCl intake per kg body weight per day between the young and old rats on the LS diet (Fig. 3). The consumption of NaCl per kg of BW increased dramatically in both age groups with the HS diet on days 1-2 and 3-6, but the young rats increased their consumption of NaCl more than the old rats (p<0.01 by two-way ANOVA). The young rats continued to increase their NaCl consumption on days 3-6 relative to days 1-2 on the HS diet (p<0.01) but the old rats did not significantly increase their NaCl consumption from days 1-2 to days 3-6.

Urine volume and osmolality

In young rats, urine volume increased by 3-fold two days after being transferred from a LS to a HS diet and remained elevated on day 6 (Fig. 4A&B). Urine volume in the old rats was similar to the young rats on day 0 of the HS diet; however, urine volume did not increase after switching to the HS diet (p<0.0001, young vs old by two-way ANOVA) (Fig. 4A).

Urine osm in 4 mo rats decreased by 33% two days after being transferred from a LS to a HS diet (Fig. 4C&D). Four days later, the urine osm was indistinguishable from day zero in the young rats (Fig. 4C&D). No significant differences in urine osmolality were detected between young and old rats maintained on a LS diet for two weeks (Fig. 4C&D). In contrast to the young animals, urine osm in the old rats was not attenuated in response to switching to a HS diet (Fig. 4D).

Plasma sodium and potassium
There were no differences in plasma sodium between the young and old rats (Fig. 5A) or in plasma potassium (Fig. 5C) between young and old rats maintained on the LS diet. There were also no detectable differences in the magnitude of the plasma sodium increase in response to the HS diet in young (1.2-fold) and old (1.2-fold) rats on day 6 (Fig. 5B).

Transferring to a HS diet lowered plasma potassium in young and old rats at two days (Fig. 5C). On day 6, plasma potassium returned to levels observed before the sodium load in the young rats. In contrast, plasma potassium remained lower than before the sodium load in the old rats (p<0.002, young vs old by two-way ANOVA) (Fig. 5D).

**Plasma aldosterone**

In the young rats, plasma aldosterone decreased by 98% two days after being transferred from a LS to a HS diet (Fig. 6A&B). Six days later, plasma aldosterone remained reduced to the same extent. Though the amount of the reduction in plasma aldosterone after switching to HS was similar in the old rats (87% by day 2 and 84% by day 6), plasma aldosterone was 45% less in the old rats compared to the young rats before switching to the HS diet.

**Plasma AVP**

In young rats, plasma AVP levels increased after six days on HS but the difference was not statistically significant (LS: 3.0 ± 0.8 pg/ml, n=4; HS: 3.9 ± 0.4, n=4). Similarly there was no significant difference in plasma AVP levels in old rats on LS (7.2 ± 1.4, n=4) versus HS (6.5 ± 1.1, n=3). Plasma AVP levels were significantly higher in old versus young animals on either the LS or HS diets (p<0.05).

**Adrenocortical AT1R density**
Radioligand binding assays on adrenocortical membranes using $^{125}\text{-I[Sar}^{1},\text{Ile}^{8}]\text{Ang II}$ revealed that the density of AT$_1$R in the adrenal cortex of young rats was decreased by 35% and 43%, respectively, on days 2 and 6 after switching to the HS diet (Fig. 7A&B). AT$_1$R densities were 29% less in the old rats before switching to the HS diet and AT$_1$R densities were not reduced as rapidly in response to a HS diet compared to the young animals ($p<0.001$, young vs. old by two-way ANOVA). In fact, a significant drop in adrenocortical AT$_1$R density was not observed until day 6 in the old rats (Fig. 7). Two-way ANOVA showed no significant differences in binding affinity as a function of age or dietary sodium and there was no interaction (the average Kd was 0.14 ± 0.02 nM).

Adrenocortical AT$_{1a}$R and AT$_{1b}$R mRNA

No differences in the AT$_{1a}$R mRNA expression levels were detected as a function of age or days on the HS diet (Fig. 8A); however, after normalizing AT$_{1a}$R mRNA expression to day zero of the HS diet, we found the ratio of HS/LS was reduced by 38% on day 2 in the young rats though no significant inhibition was observed on day 6 (Fig. 8B). There was no effect of the HS diet on AT$_{1a}$R mRNA levels or on the ratio of HS/LS in the old rats (Fig. 8A&B).

AT$_{1b}$R mRNA expression was 48% less in the old rats compared to the young rats before switching to the HS diet ($p<0.05$, by two-way ANOVA) (Fig. 8C). Expression of the AT$_{1b}$R mRNA was decreased in the young rats by 39% and 46%, respectively, on days 2 and 6 after transferring to the HS diet (Fig. 8C). While transferring to the HS diet showed a trend to decrease AT$_{1b}$R mRNA in the old rats on day 2, this effect did not reach statistical significance even after normalizing the data to LS levels (Fig. 8C & 8D).

Discussion

The main findings of this study are that aging impaired the adrenal AT$_1$R response to
a dietary sodium load in male Fischer rats. Adrenal AT₁R densities and AT₁bR mRNA were
29% and 48% less, respectively, in old rats before the sodium load and AT₁R densities
were not reduced as rapidly in response to a HS diet compared to the young animals.
These age-associated effects on adrenal AT₁R densities and AT₁bR mRNA levels
correlated with reduced water intake and plasma aldosterone with little change in urine
volume, urine osmolality or plasma AVP.
In response to an increase in dietary sodium, urine volume increases in an effort to
rid the body of excess sodium; however, this ability to increase urine volume is impaired
in the elderly (35). These findings in humans are also observed in experimental
models of aging. Previous studies have shown that an intracarotid injection of hypertonic
sodium chloride resulted in a blunted antidiuretic response in the old rat (20 mo)
compared to the young (< 6 mo) male (19, 51). Our study in male Fischer 344/BN rats
extends these findings by demonstrating that in response to a dietary sodium load,
compared to the young rats, the old animals exhibited a diminished ability to rapidly
increase water intake (Fig. 2A&B) and urine volume (Fig. 4A&B), which resulted in a
decreased ability to rapidly lower urine osmolality (Fig. 4C&D) and maintain plasma
potassium homeostasis (Fig.4C&D). This diminished ability to handle a dietary sodium load
may have contributed to the greater reduction in NaCl intake (mg/kg BW) in the old rats
relative to the young rats (Fig. 3).
Our findings support previous studies in male Fischer 344 rats subjected to
dehydration. Maximum urine electrolyte concentration after 40 h of dehydration was
significantly lower in old, 23 mo rats compared to 4 mo rats (12). Furthermore, the
fraction of infused sodium excreted during and after expansion with isotonic saline was
attenuated in old (22-24 mo) compared to the young (4-6 mo) rats (11). Similar findings
of an impaired ability to excrete sodium with volume expansion are observed in humans. Elderly men placed on a sodium restricted diet had lower urine osmolality than young men (36).

Previous studies have shown that aging alters aldosterone metabolism. While aging had little effect on plasma aldosterone on an unrestricted sodium diet, men and women greater than 50 years of age had markedly lower plasma aldosterone levels on a LS diet compared to those who were 20 to 30 years old (23). Furthermore, urinary aldosterone excretion in 70-90 year old men was significantly less than found in 18-28 year old men (21). Consistent with this clinical research, we found that plasma aldosterone in the old rats maintained for 2 weeks on a LS diet was nearly half the level found in the young rats (Fig. 6).

Not only did we observe lower levels of plasma aldosterone in the old rats on a sodium restricted diet, we also found the density of adrenocortical AT1R were markedly lower in the old rats compared to the young animals (Fig. 7). We previously demonstrated a positive correlation exists between the modulation of adrenal AT1R density and plasma aldosterone; a 30% reduction in adrenocortical AT1R density induced by 17β-estradiol in ovariectomized female rats was associated with significant reductions in plasma aldosterone (52). Taken together, our findings suggest the age-associated decline in plasma aldosterone is due to reduced adrenocortical AT1R densities. This observation supports previous studies showing that the adrenal zona glomerulosa in humans (42, 44), rats (9, 20) and cows (49) undergoes an age-dependent impairment in its aldosterone secretory response to Ang II. While our study was conducted solely in male rats, we expect adrenocortical AT1R densities would also be decreased in old female Fischer 344/BN rats since aging (22-24 mo) in female Long-Evans rats was associated with diminished aldosterone secretion in response to Ang II (50).
In contrast to our findings in the adrenal cortex, a previous study in Fisher 344 rats reported higher immunoreactive AT$_1$R protein expression in the adrenal medulla in 24 mo old rats compared to young adult animals (17). However, this previous study measured immunoreactive AT$_1$R protein expression by Western blot rather than receptor density by radioligand binding and several studies have challenged the specificity of available AT$_1$R antibodies (1, 13, 24). Thus, it is possible that immunoreactive AT$_1$R protein expression does not correlate with receptor density due to antibody non-specificity, post-translational regulation and/or because of discordant regulation of the AT$_1$R in the adrenal cortex and adrenal medulla. Moreover, it is important to note that the AT$_1$R that mediates aldosterone secretion is located in the zona glomerulosa cells and not the adrenal medulla. The adrenal cortex is not the only tissue reported to exhibit an age-related decline in AT$_1$R density. AT$_1$R densities determined by autoradiography were found to be diminished by more than 50% in the paraventricular nucleus and by approximately 35% in the organum vasculosum laminae terminalis in 20 mo Fischer 344 rats compared to 5-15 mo rats (53).

Ang II (via its actions on AT$_1$Rs in the adrenal cortex) is the major regulator of plasma aldosterone during altered sodium intake. Early studies showed that adrenal AT$_1$Rs are up-regulated by Ang II and sodium restriction (4-6). Thus, the age-related reduction in adrenocortical AT$_1$Rs is likely a response to decreased plasma Ang II as a function of aging. While we had insufficient sample material to measure plasma Ang II in this study, reports in male rats have shown that there is an age-related decrease in the rate limiting step in Ang II formation, i.e., plasma renin activity (37) as well as serum angiotensin converting enzyme activity (41). Furthermore, plasma Ang II and plasma renin activity are both lower in the elderly compared to young individuals (43). Studies have also shown that the dipsogenic response to Ang II administered subcutaneously was more robust in rats at 3 mo of age compared to 12, 20 or 24 mo of age (58).
Aging is associated with reduced plasma potassium levels (46) and lower fractional excretion of potassium in men and women (42). The elderly are at greater risk for impaired potassium homeostasis than young adult (48). The AT_1R plays a critical role in maintaining plasma potassium levels. In this study, we found that the HS diet substantially reduced adrenal AT_1R densities after switching from the LS to the HS diet in both young and old rats; however, young rats were able to reduce the density of adrenocortical AT_{1R}s within 48 h while old rats took at least 6 days to achieve the same magnitude effect (Fig. 7). These data suggest that aging impairs the ability to rapidly down-regulate the AT_1R in response to an increase in dietary sodium. Therefore, impairment in adrenocortical AT_1R regulation could contribute to the reduced ability of the elderly to respond appropriately to a sodium load. Furthermore, the attenuation of rapid AT_1R regulation was coincident with the impairment in urine concentrating ability and the finding that plasma potassium fell in response to increased dietary sodium in old but not young rats (Fig. 5C&D). Taken together, these findings suggest the slower ability of adrenal AT_{1R}s to down-regulate in response to sudden increases in dietary sodium results in a more sluggish water intake and urine concentrating response and subsequent impaired potassium homeostasis.

There are two subtypes of the AT_1R (AT_{1aR} and AT_{1bR}) in rats and mice. These receptor subtypes share 95% amino acid homology (57) and there are no currently available pharmacological agents that can effectively differentiate between their protein expression; however, the mRNA levels of these receptor subtypes can be distinguished (33). Studies have shown that the AT_{1aR} mRNA is widely expressed throughout rodent tissues and in general, is far more abundant than the AT_{1bR} mRNA except in a few tissues including the adrenal cortex where PCR amplification (27, 59) and in situ hybridization (26) showed the majority (80%) of adrenal AT_{1R} is of the AT_{1bR} subtype. Therefore, we expect
the adrenal AT_{1b}R plays a greater role in the response of the adrenal AT_{1}R to a sodium
load than the AT_{1a}R subtype; however, we cannot rule out that the minority population of
adrenal AT_{1a}Rs also contributed to the age-associated dysregulation of adrenal AT_{1}Rs.

Old rats express 48% less AT_{1b}R mRNA in the adrenal cortex than the young rats
under conditions of sodium restriction. Therefore, the lower density of adrenocortical
AT_{1}Rs is likely the result of an age-related reduction in the transcription of the AT_{1b}R mRNA
since lower mRNA levels could lead to less mRNA translation into AT_{1b}R protein. As a
consequence, fewer AT_{1b}R would be available in the old rat adrenal cortex to stimulate
aldosterone secretion in response to Ang II. This age effect on plasma aldosterone would
be magnified under LS conditions since Ang II levels would be elevated and Ang II-induced
aldosterone secretion would be increased compared to high dietary sodium conditions in
which Ang II levels are suppressed and aldosterone secretion is maximally inhibited (3).
In contrast, the AT_{1a}R subtype is not likely to contribute to age effects on adrenal AT_{1}R
function including aldosterone secretion since no differences were observed in the mRNA
expression of the AT_{1a}R transcript in the adrenal cortex between young and old rats on a
LS diet. This differential regulation of the two subtypes is not surprising given that these two
receptor subtypes have been shown to be dis-coordinately regulated by dietary sodium in
other tissues including the brains of adult rats (55) and mice (15), suggesting that
tissue-specific receptor subtype regulation occurs.

Our finding that the HS more rapidly down-regulates the AT_{1b}R transcript in young
compared to old rats and strongly correlates with changes in AT_{1}R densities in the young
and old animals suggests that impairment in the transcriptional regulation of the AT_{1b}R
contributes to the age-associated defect in AT_{1}R regulation in response to increased
dietary sodium. Decreased mRNA expression levels could also reflect increased receptor
mRNA turnover since receptor expression is known to be regulated at the level of mRNA
stability. For example, Ang II down-regulates AT₁R densities in vascular smooth muscle cells by decreasing mRNA stability (31).

Aging blunts thirst and alters AVP responsiveness to physiologically relevant stimuli (8). Although originally controversial, it has now been demonstrated in numerous studies that in rodents and man plasma AVP increases with aging and indeed aging has been characterized as a state of relative AVP resistance (8, 39). Our results (Fig. 2A&B) support prior findings and demonstrate not only an age-associated decrease in water intake, but also a blunted drinking response to HS diet. Additionally, our results indicate that AVP levels while elevated in old animals compared to young animals do not change in response to a dietary sodium load. Although much more work is needed, in particular a detailed examination of the time course of potential changes in plasma AVP as animals adjust to the increased salt intake, our preliminary data suggest that in the case of the young rats, renal mechanisms accommodate the increased salt load so that there is no stimulus for additional AVP secretion. In old animals, on the other hand, it is possible that AVP secretion is already maximally elevated and the switch to HS fails to alter the already activated state. It would be interesting as well to determine at the cellular level whether production of AVP and the other neurohypophysial hormone, oxytocin, changes with HS in young versus old rats. Oxytocin is known to exert anorexigenic actions in rodents (45) and central release of oxytocin has been demonstrated in rats following osmotic stimuli (29, 61). If oxytocin released centrally in response to HS in our animals is the reason for the maintained suppression of food intake in the old rats as in the young animals, then unlike AVP, it would not appear that aging is an oxytocin-resistant state. There is evidence that unlike AVP, plasma levels of oxytocin do not significantly differ between 3 and 32 mo old rats (18).

We did not measure blood pressure in this study; however, the male Fischer 344/BN rat is
not a model of dietary sodium-induced hypertension. Chugh et al. (16) showed in young (2 mo) and old (20 mo) male Fischer 344/BN rats that a HS (8% NaCl) diet for 4 weeks increased systolic blood pressure by less than 5 mm Hg. Therefore, it is unlikely that the age-associated effects were due to hypertension.

**Perspectives and Significance**

In conclusion, the age-related decline in AT$_{1b}$Rs in the adrenal cortex contributes to reduced water intake and plasma aldosterone levels during conditions of sodium restriction and dysregulation of AT$_1$R-mediated responses to sodium loading including water intake, urine concentrating ability and potassium homeostasis. These findings suggest that dysregulation of adrenocortical AT$_{1b}$Rs in response to dietary sodium manipulation contributes to the defects in water and electrolyte homeostasis observed in the old Fischer male rat. These findings may have clinical implications for dietary sodium consumption by the elderly and suggests this population could be more susceptible to the adverse consequences of a HS diet.

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**Disclosures**

None

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Figure legends

Fig. 1 Effect of age on body weight changes in response to a high sodium diet. Body weight (g) is expressed as (A) mean ± SEM or (B) HS/LS ratio on days 0, 2 and 6 after the onset of a HS diet; *p<0.05 vs day 0 within same age groups; #p<0.05 vs young rats on same day; n=6-8/group.

Fig. 2 Effect of age on water and food intake in response to a high sodium diet. (A, B) Water or (C, D) food intake were normalized to BW and the results are expressed as (A, C) mean ± SEM or (B, D) HS/LS ratio on days 0, 2 and 6 after the onset of a HS diet; *p<0.05 vs day 0 within same age groups.

Fig. 3 Effect of age on dietary sodium intake in response to a high sodium diet. Dietary sodium intake was normalized to BW and the results are expressed as mean ± SEM on days 0, 2 and 6 after the onset of a HS diet; *p<0.05 vs day 0 within same age groups; #p<0.05 vs young rats on same day; n=6/group.

Fig. 4 Effect of age on urine volume and osmolality in response to a high sodium diet. (A, B) Urine volume or (C, D) urine osmolality were normalized to BW and the results are expressed as (A, C) mean ± SEM or (B, D) HS/LS ratio on days 0, 2 and 6 after the onset of a HS diet; *p<0.05 vs day 0 within same age groups; #p<0.05 vs young rats on same day; n=7-8/group.

Fig. 5 Effect of age on plasma sodium and potassium in response to a high sodium diet. (A,
B) Plasma sodium or (C, D) plasma potassium are expressed as (A, C) mean ± SEM or (B, D) HS/LS ratio on days 0, 2 and 6 after the onset of a HS diet; *p<0.05 vs day 0 within same age groups; #p<0.05 vs young rats on same day; n=7-8/group.

Fig. 6 Effect of age on plasma aldosterone in response to a high sodium diet. Plasma aldosterone is expressed as (A) mean ± SEM or (B) HS/LS ratio on days 0, 2 and 6 after the onset of a HS diet; *p<0.05 vs day 0 within same age groups; #p<0.05 vs young rats on same day; n=7-8/group.

Fig. 7 Effect of age on adrenocortical AT1R density in response to a high sodium diet. AT1R densities are expressed as (A) mean ± SEM or (B) HS/LS ratio on days 0, 2 and 6 after the onset of a HS diet; *p<0.05 or **p<0.01 vs day 0 within same age groups; #p<0.05 vs young rats on same day; n=7-8/group.

Fig. 8 Effect of age on AT1aR and AT1bR mRNA expression in response to a high sodium diet. (A, B) AT1aR or (C, D) AT1bR mRNA are expressed as (A, C) mean ± SEM or (B, D) HS/LS ratio on days 0, 2 and 6 after the onset of a HS diet; *p<0.05 vs day 0 within same age groups; #p<0.05 vs young rats on same day; n=6-8/group.
Days of HS | Urine volume (mL/Kg BW) | Urine osmolality (mosm/kg H$_2$O)
---|---|---
0 | 0.0 | 0.0
2 | 0.9 | 0.4
6 | 1.8 | 0.8

Days of HS | Urine volume (ratio of HS/LS) | Urine osmolality (ratio of HS/LS)
---|---|---
0 | 0.0 | 0.0
2 | 2.7 | 0.9
6 | 3.6 | 1.2

A. Urine volume (mL/Kg BW) for Young and Old groups over 6 days of HS.
B. Urine volume (ratio of HS/LS) over 6 days of HS.
C. Urine osmolality (mosm/kg H$_2$O) for Young and Old groups over 6 days of HS.
D. Urine osmolality (ratio of HS/LS) over 6 days of HS.
A. Plasma Aldosterone (pg/ml)

B. Plasma Aldosterone (Ratio of HS/LS)

Days of HS

- Young
- Old

* Indicates statistical significance

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Plasma Aldosterone (pg/ml)

Plasma Aldosterone (Ratio of HS/LS)
**A**

AT$_1$R $B_{\text{max}}$ (fmol/mg protein)

Young

Old

**B**

AT$_1$R $B_{\text{max}}$ (Ratio of HS/LS)

Days of HS

0 2 6 0 2 6